Title

Concentration and synthesis of phosphoribosylpyrophosphate in erythrocytes from normal, hyperuricemic, and gouty subjects.

Journal

Metabolism: clinical and experimental, 20(8)

ISSN

0026-0495

Authors

Meyskens, FL
Williams, HE

Publication Date

1971-08-01

DOI

10.1016/s0026-0495(71)80003-3

Concentration and Synthesis of Phosphoribosylpyrophosphate in Erythrocytes From Normal, Hyperuricemic, and Gouty Subjects

By FRANK L. MEYSKENS AND HIBBARD E. WILLIAMS

Phosphoribosylpyrophosphate (PRPP) synthetase activity and the intracellular concentration of PRPP were assayed in erythrocytes from patients with primary hyperuricemia and primary metabolic gout. Sensitivity of the enzyme to feedback inhibition by adenosine diphosphate (ADP), guanosine diphosphate (GDP), and 2,3-diphosphoglycerate (2,3-DPG) was determined. All patients with gout and four of ten patients with hyperuricemia were taking allopurinol during the study. Mean PRPP synthetase activity in erythrocytes from hyperuricemic and gouty patients was similar to that in normal subjects, and feedback inhibition by ADP, GDP, and 2,3-DPG was intact. The concentration of PRPP in erythrocytes was higher in normal females than in normal males, higher in normal subjects than in gouty patients, and lower in hyperuricemic patients taking allopurinol than in those hyperuricemic patients not taking this drug. The difference in intracellular levels of PRPP in erythrocytes in gout versus hyperuricemic patients was not significant. The significance of these findings is discussed in relation to the regulation of PRPP synthetase and in the important regulatory role of PRPP in purine metabolism.

THE INTRACELLULAR CONCENTRATION of 5-phosphoribosyl-1-pyrophosphate (PRPP) appears to be important in the regulation of purine metabolism.[1-4] Altered intracellular levels of PRPP have been reported in individuals with absence of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRTase)[2,5] and in individuals with primary metabolic gout with normal HGPRTase levels.[2,6] Recently depletion of erythrocyte PRPP levels have been reported in hyperuricemic subjects given allopurinol.[7] Because abnormal biosynthesis of PRPP might contribute to the pathogenesis of some cases of primary metabolic gout, the intracellular concentration of PRPP and the synthesis of PRPP and its regulation by feedback inhibition were studied in erythrocytes from normal subjects, patients with primary hyperuricemia, and patients with primary metabolic gout.

MATERIALS AND METHODS

Ten patients were considered to have primary hyperuricemia (mean age 49 yr) based on

From the Medical Services, San Francisco General Hospital, and the Department of Medicine, University of California, San Francisco, Calif.

Received for publication January 28, 1971.

Supported by USPHS Grant AM-13672.

FRANK L. MEYSKENS, *B.S.: Third Year Medical Student, University of California, San Francisco, Calif.* HIBBARD E. WILLIAMS, M.D.: *Chief of Medical Services, San Francisco General Hospital; Associate Professor of Medicine, University of California School of Medicine, San Francisco, Calif.*

serum uric acid concentrations (7.0–9.5 mg/100 ml) in the upper 2% of the population, normal creatinine clearance values, and no clinical evidence of gout, hematologic abnormalities, or malignant disease. Seven patients with primary metabolic gout (mean age 52 yr) had hyperuricemia of unknown origin and either a clinical history of gout or evidence of subcutaneous tophaceous deposits. Uric acid levels in the gouty patients ranged from 7.2–11.5 mg/100 ml. Overproduction of uric acid was not established in these patients. Four of ten hyperuricemic patients and all gouty subjects were receiving allopurinol (dose range 100–400 mg/day) during the study. The control group consisted of ten volunteers (mean age 28 yr) with serum uric acid concentrations within the normal range (3–6.0 mg/100 ml). All subjects had normal erythrocyte HGPRTase levels.

Nonfasting blood samples were drawn by venipuncture into heparinized tubes and chilled (4°C) immediately. The activity of PRPP synthetase was determined by the two-step procedure of Hershko et al., using a charcoal-treated erythrocyte hemolysate in all assays.[1] Inhibition of PRPP synthetase by adenosine diphosphate (ADP), guanosine diphosphate (GDP), and 2,3-diphosphoglycerate (2,3-DPG) was determined by addition of these compounds to the reaction mixture at concentrations of 0.125, 0.125, and 5.0 m*M*, respectively. Inorganic phosphate (P_i) was maintained at a concentration of 5 m*M*, and an ATP-regenerating system was used in all assays. The formation of PRPP was found to be linear to enzyme concentration and time at the concentrations of ATP, P_i, and ribose-5-phosphate used. The concentration of PRPP was assayed by the method of Henderson and Khoo,[8] using hypoxanthine as substrate. This method is based on the observation that the concentration of PRPP is limiting in the conversion of hypoxanthine to inosinic acid by HGPRTase. Heparinized blood, chilled (4°C) immediately and washed twice with two volumes of 0.9% saline, was centrifuged at 10,000 *g* for 15 min. Ethylenediaminetetraacetic acid (EDTA), 1.0 ml of 1 m*M*, was added to 1.0 ml of the packed erythrocytes. The EDTA-stabilized mixture was then heated for 90 sec in boiling water and chilled (4°C) immediately. 10% Norit A charcoal, 0.10 ml, was added to adsorb endogenous nucleotides. After mixing, the charcoal was deposited by centrifugation at 10,000 *g* for 10 min. Aliquots of the supernatant were assayed for PRPP using a dialyzed erythrocyte hemolysate with an HGPRTase activity of 100 mμmoles/hr per mg protein. The HGPRTase activity was determined by the method of Kelley et al.[9] Radioactive hypoxanthine, inosine, and inosinic acid were separated by high voltage electrophoresis with a Savant Electrophorator (0.05 *M* borate buffer, pH 9.0, 3,000 V for 1 hr). Protein was determined by the method of Lowry et al.[10]

Reduced glutathione (GSH), adenosine triphosphate (ATP), ribose-5-phosphate (R5P), ADP, GDP, 2,3-DPG, phosphoenolpyruvate (PEP), and pyruvate kinase (PK) were purchased from Sigma Biochemical Co. Hypoxanthine-8-C^{14} (54.3 mCi/mmole) was purchased from New England Nuclear Corp. as the crystalline solid and was dissolved in distilled water prior to use to give a final concentration of 10 μCi/ml.

RESULTS

The intracellular concentration of erythrocyte PRPP varied among the different groups studied (Table 1). Normal subjects had a mean level of 3.03 ±

Table 1.—Concentration of PRPP in Erythrocytes of Normal Subjects and Hyperuricemic and Gouty Patients

	Erythrocyte PRPP Concentration* (mμmoles/ml Packed Erythrocytes)		
Sex	Normal Subjects	Hyperuricemic Patients	Gouty Patients
Female	3.29 ± 0.47 (5)	3.15 ± 0.31 (3)	2.92 (1)
Male	2.77 ± 0.17 (5)	3.30 ± 0.55 (7)	2.26 ± 0.40 (6)
Mean	3.03 ± 0.32 (10)	3.22 ± 0.38 (10)	2.33 ± 0.39 (7)

* Mean ± SD; numbers in parentheses are number of subjects.

Table 2.—PRPP Synthetase Activity in Erythrocyte Lysates and % Inhibition of Synthetase in Presence of ADP, GDP, and 2,3-DPG

Subjects	Synthetase Activity* (mμmoles/hr/mg protein)	% Synthetase Inhibition		
		0.125 m*M* ADP	0.125 m*M* GDP	5.0 m*M* 2,3-DPG
Normal	32.0 ± 5.3	72	64	80
Hyperuricemic	32.7 ± 5.9	70	74	85
Gouty	34.1 ± 6.1	64	75	76

* Mean ± SD.

0.32, hyperuricemic patients, 3.22 ± 0.38, and gouty patients, 2.33 ± 0.39 mμmoles/ml packed erythrocytes. The difference in the concentration of PRPP in both the hyperuricemic and normal subjects compared with the levels of PRPP in gouty patients was significant at $p < 0.05$ (Student's t test). Normal female subjects had a higher level of erythrocyte PRPP than the normal males [3.29 ± 0.47 versus 2.77 ± 0.17 mμmoles/ml packed erythrocytes ($p < 0.05$)]. The mean erythrocyte PRPP level in the four hyperuricemic patients receiving allopurinol was 2.94 ± 0.29, compared with 3.47 ± 0.30 mμmoles/ml packed erythrocytes in the six patients not taking the drug ($p < 0.05$). The effect of diet on PRPP levels was not determined in this study, although administration of large doses of both purines and pyrimidines have been shown to effect intracellular PRPP levels.[3,4]

The activity of PRPP synthetase in erythrocyte hemolysates from normal, hyperuricemic, and gouty subjects was 32.0 ± 5.3, 32.7 ± 5.9, and 34.7 ± 6.1 mμmoles/hr per mg protein, respectively. Inhibition of PRPP synthetase by ADP, GDP, and 2,3-DPG was similar in all three groups (Table 2).

No correlation could be demonstrated between the concentration of erythrocyte PRPP and the activity of PRPP synthetase.

Discussion

Recent studies clearly demonstrate a partial deficiency of the enzyme HGPRTase in a small percentage of patients with primary metabolic gout.[9] In two patients evidence exists for an increase in uric acid production secondary to decreased feedback inhibition of the enzyme PRPP amidotransferase.[4] However, the basic pathogenetic mechanisms operative in most cases of gout and hyperuricemia secondary to increased production of uric acid remain obscure. Recently, emphasis has been placed on the intracellular concentration of PRPP as an important factor controlling the production of uric acid.[11,12] This compound is utilized at several sites in the synthetic pathways of purines and nucleotides (Fig. 1). An increase in PRPP synthesis leading to increased intracellular levels of this compound might result in increased production of purines. This is supported by the observation that intracellular levels of PRPP are well below the reported K_m of this compound for the enzyme PRPP amidotransferase, the rate-limiting reaction in the de novo pathway for purine synthesis.[13-16]

The normal activity of PRPP synthetase observed in erythrocytes from hyperuricemic and gouty patients suggests that the overproduction of uric acid in these patients is not due to an increase in PRPP formation secondary to an abnormality in this enzyme. Likewise, normal inhibition of erythrocyte PRPP

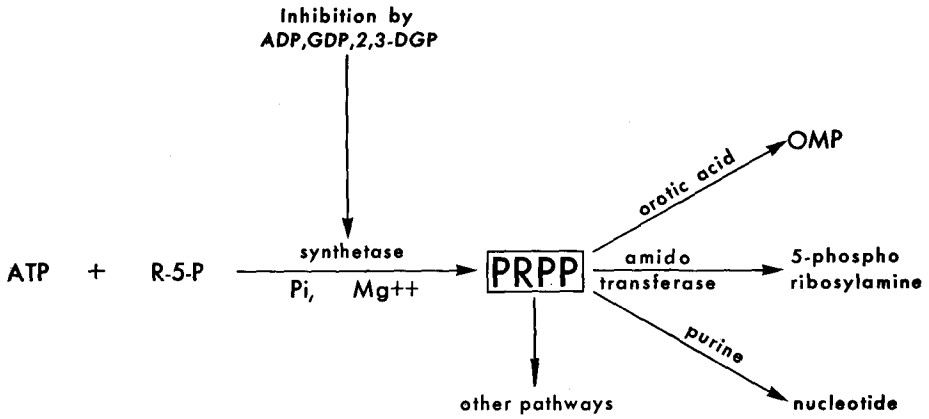

Fig 1.—Factors controlling intracellular PRPP concentration.

synthetase by ADP, GDP, and 2,3-DPG suggests further that an increase in PRPP could not be afforded by this route. These studies do not rule out a defect in PRPP synthesis in other organs, since it was not possible to determine the activity of PRPP synthetase in other tissues from these subjects. There are no data at present to suggest that this enzyme exists in isozymic forms, and the inhibition studies tend to confirm the similarity between the erythrocyte enzyme and that in other tissues.[17-19]

The de novo synthesis of PRPP is controlled by the enzyme PRPP synthetase. Although studies of this enzyme are few,[17-19] the following characteristics have been noted: ATP and R5P are substrates, high levels of P_i and divalent cation (Mg^{++} or Mn^{++}) are required, and a wide range of nucleotides inhibit the enzyme. Hershko et al. recently delineated the important role of P_i in human erythrocytes as a regulating factor in the expression of feedback inhibition and as an obligatory requirement before R5P levels become critical in the regulation of PRPP synthetase.[1]

The lack of correlation between PRPP levels and PRPP synthetase activity in the normal, hyperuricemic, and gouty subjects emphasizes the multitude of factors influencing intracellular PRPP concentrations (Fig. 1). An alteration in the hexose monophosphate shunt could provide excess R5P. An increased supply of R5P has been postulated to be a contributing factor in the hyperuricemia associated with glycogen storage disease, type I.[14] Hershko et al. noted an increased rate of formation of PRPP secondary to an elevated endogenous supply of R5P in some patients with gout.[20] An increase in ribonucleoside breakdown might provide excess R5P and might also increase purine catabolism. One study suggested that an alteration in glutathione reductase may be important in the pathogenesis of some cases of gout.[21]

The levels of PRPP measured in erythrocytes agree well with those reported in other studies.[2,7] Although PRPP concentrations in erythrocytes from primary gout patients with normal HGPRTase levels were decreased, other authors found PRPP concentrations in cultured skin fibroblasts of some gout patients were elevated when compared with the values for normal subjects.[5,6] This difference

could be due to either the presence of a de novo pathway for purine synthesis in fibroblasts, or to an effect of allopurinol in the gouty and hyperuricemic patients. The decreased levels of PRPP in erythrocytes in the gouty patients and in those hyperuricemic subjects taking allopurinol strongly suggest an effect of allopurinol on PRPP levels. Because all of the gouty subjects studied were taking allopurinol it is possible that the drug masked an abnormally elevated level of PRPP in this group. Preliminary investigation reveals that 1 m*M* allopurinol in vitro does not inhibit PRPP synthetase activity in charcoal-treated erythrocyte lysates.

Recently Fox et al.[7] demonstrated a significant depletion of erythrocyte PRPP levels in nine hyperuricemic patients given allopurinol. In this study no difference in erythrocyte PRPP concentration was found between hyperuricemic patients and normouricemic control subjects before allopurinol administration. These investigators also demonstrated a lowering of erythrocyte PRPP levels by allopurinol when studied in vitro. In addition studies in vitro using erythrocyte lysates lacking HGPRTase activity indicated that neither allopurinol nor its ribonucleotide had an effect on PRPP synthesis.

Although an alteration in PRPP synthetase activity in all subjects was not detected, the central role of PRPP in purine metabolism suggests that any alteration leading to the overproduction of PRPP might lead to overproduction of uric acid. The detection of an individual with an alteration in the metabolism of PRPP might greatly hasten and enhance the study of the regulation of PRPP and the elucidation of its role in purine metabolism.

ACKNOWLEDGMENT

The authors thank Dr. Jeffrey Fessel and Dr. Benedict Budai of the Kaiser-Permanente Foundation Hospital, San Francisco, for allowing us to study their patients with hyperuricemia and gout; and Dr. Charles Epstein of the University of California, San Francisco, for the use of the Savant Electrophorator.

REFERENCES

1. Hershko, A., Razin, A., and Mager, J.: Regulation of the synthesis of 5-phosphoribosyl-1-pyrophosphate in intact red blood cells and in cell-free preparations. Biochim. Biophys. Acta 184:64, 1969.

2. Greene, M. L., and Seegmiller, J. E.: Erythrocyte 5-phosphoribosyl-1-pyrophosphate (PRPP) in gout: importance of PRPP in regulation of human purine synthesis. Arthritis Rheum. 12:666, 1969.

3. Kelley, W. N., Greene, M. L., Rosenbloom, F. M., Wyngaarden, J. B., and Seegmiller, J. E.: Effects of orotic acid on purine biosynthesis in vivo and in vitro. Arthritis Rheum. 12:673, 1969.

4. —, Fox, I. H., and Wyngaarden, J. B.: Essential role of phosphoribosylpyrophosphate (PRPP) in regulation of purine biosynthesis in cultured human fibroblasts. Clin. Res. 18:457, 1970.

5. Rosenbloom, F. M., Henderson, J. F., Caldwell, I. C., Kelley, W. N., and Seegmiller, J. E.: Biochemical bases of accelerated purine biosynthesis *de novo* in human fibroblasts lacking hypoxanthine-guanine phosphoribosyltransferase. J. Biol. Chem. 243:1166, 1968.

6. Henderson, J. F., Rosenbloom, F. M., Kelley, W. N., and Seegmiller, J. E.: Variations in purine metabolism of cultured skin fibroblasts from patients with gout. J. Clin. Invest. 47:1511, 1968.

7. Fox, I. H., Wyngaarden, J. B., and Kelley, W. N.: Depletion of erythrocyte phosphoribosylpyrophosphate in man. A newly observed effect of allopurinol. New. Eng. J. Med. 283:1177, 1970.

8. Henderson, J. F., and Khoo, M. K. Y.: Synthesis of 5-phosphoribosyl-1-pyrophosphate from glucose in Ehrlich ascites tumor

cells *in vitro*. J. Biol. Chem. 240:2349, 1965.

9. Kelley, W. N., Rosenbloom, F. M., Henderson, J. F., and Seegmiller, J. E.: A specific enzyme defect in gout associated with overproduction of uric acid. Proc. Nat. Acad. Sci. (USA) 57:1735, 1967.

10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265, 1951.

11. Seegmiller, J. E.: Diseases of purine and pyrimidine metabolism. *In* Bondy, P. K., and Rosenberg, L. E. (Eds.): Duncan's Diseases of Metabolism, Vol. I (ed. 6). Philadelphia, W. B. Saunders, 1969, p. 576.

12. Rajalakshmi, S., and Handschumacher, R. E.: Control of purine biosynthesis de novo by orotic acid in vivo and in vitro. Biochim. Biophys. Acta 155:317, 1968.

13. Wyngaarden, J. B., and Ashton, D. M.: The regulation of activity of phosphoribosylpyrophosphate amidotransferase by purine ribonucleotides: A potential feedback control of purine biosynthesis. J. Biol. Chem. 234:1492, 1959.

14. Henderson, J. F., and Khoo, M. K. Y.: On the mechanism of feedback inhibition of purine biosynthesis de novo in Ehrlich ascites tumor cells in vitro. J. Biol. Chem. 240: 3104, 1965.

15. Nierlich, D. P., and Magasanik, B.: Regulation of purine ribonucleotide synthesis by end product inhibition: The effect of adenine and guanine ribonucleotides on the 5′-phosphoribosylpyrophosphate amidotransferase of *Aerobacter aerogenes*. J. Biol. Chem. 240:358, 1965.

16. Hill, D. L., and Bennett, L. L., Jr.: Purification and properties of 5-phosphoribosyl pyrophosphate amidotransferase from adenocarcinoma 755 cells. Biochemistry (Wash.) 8:122, 1969.

17. Atkinson, D. E., and Fall, L.: Adenosine triphosphate conservation in biosynthetic regulation: *Escherichia coli* phosphoribosylpyrophosphate synthase. J. Biol. Chem. 242: 3241, 1967.

18. Wong, P. C. L., and Murray, A. W.: 5-Phosphoribosyl pyrophosphate synthetase from Ehrlich ascites tumor cells. Biochemistry (Wash.) 8:1608, 1969.

19. Switzer, R. L.: Regulation and mechanism of phosphoribosylpyrophosphate synthetase: I. Purification and properties of the enzyme from *Salmonella typhimurium*. J. Biol. Chem. 244:2854, 1969.

20. Hershko, A., Hershko, C., and Mager, J.: Increased formation of 5-phosphoribosyl-1-pyrophosphate in red blood cells of some gouty patients. Israel J. Med. Sci. 4:939, 1968.

21. Long, W. K.: Glutathione reductase in red blood cells: variant associated with gout. Science 155:712, 1967.